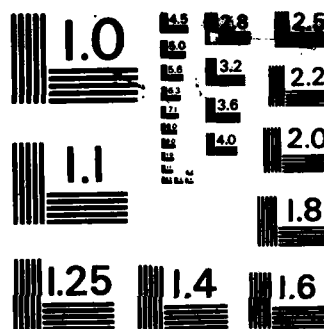MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A

TECHNICAL NOTE NO. 83-45TN

COMPUTER-ASSISTED STATISTICAL ANALYSIS.
III. The *Drosophila melanogaster* **Sex-Linked Recessive Lethal Assay**

NELSON R. POWERS, PhD, CPT MSC
and
WARREN W. JEDERBERG, MS, CPT MSC

TOXICOLOGY GROUP
DIVISION OF RESEARCH SUPPORT

AUGUST 1983   Toxicology Series 61

LETTERMAN ARMY INSTITUTE OF RESEARCH
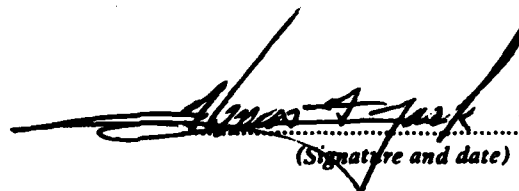**PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129**

Computer-Assisted Statistical Analysis. III. The <u>Drosophila</u> <u>melanogaster</u>
Sex-Linked Recessive Lethal Assay (Toxicology Series 61)--Powers and
Jederberg

This material has been reviewed by Letterman Army Institute
of Research and there is no objection to its presentation and/
or publication. The opinions or assertions contained herein
are the private views of the author(s) and are not to be con-
strued as official or as reflecting the views of the Department
of the Army or the Department of Defense. (AR 360-5)

(Signature and date)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Technical Note No. 83-45TN	2. GOVT ACCESSION NO. AD-A142 927	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Computer-Assisted Statistical Analysis. III. The Drosophila melanogaster Sex-Linked Recessive Lethal Assay		5. TYPE OF REPORT & PERIOD COVERED Final Dec 79 – Dec 80
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Nelson R. Powers, PhD, CPT MSC Warren W. Jederberg, MS, CPT MSC		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS A5 3M167701871 WU 203
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE August 1983
		13. NUMBER OF PAGES 16
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

THIS DOCUMENT HAS BEEN APPROVED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Drosophila melanogaster, Sex-Linked Recessive Lethal Assay, Computer-Assisted Statistical Analysis, Automated Data Analysis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report contains computer programs which summarize the data. One program calculates the mean mutation frequency with its variance expressed as a percent and the other executes statistical analyses of the data obtained from the Drosophila melanogaster Sex-Linked Recessive Lethal Assay.

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE

84 07 06 002

PREFACE

This technical note is the third in a series on the utilization of the computer to assist mutagenicity testing at Letterman Army Institute of Research. In this report we detail three functions: searching of a specific file to summarize the data; calculating the mean mutation frequency with its variance expressed as percentages; and using a statistical program for analysis of the data. Using the program in the Drosophila melanogaster Sex-Linked Recessive Lethal Assay provides an easy manner to view the data and perform statistical analyses.

i

Computer-Assisted Statistical Analysis III.
The Drosophila melanogaster Sex-Linked
Recessive Lethal Assay

The Drosophila melanogaster Sex-Linked Recessive Lethal Assay requires the testing of 8000 to 10,000 X-chromosomes for each compound and concurrent negative control in addition to 500 X-chromosomes for a positive control. A mass of data must be processed. Therefore, two computer programs were developed to summarize the results. By totalling the number of lethal mutations and non-lethal mutations for each brood of each replicate yielding the total number of lethal mutations and number of tests, this first program calculates the percentage mean mutation frequency and its variance. The second program performs the statistical analysis using the BMDP statistical software[TM] package by testing the null hypothesis if the number of lethal mutations resulting from the compound is non-significantly from different from the number resulting from the negative control.

DESCRIPTION OF THE PROGRAM

The program DRUNSUM.FR (Appendix A) searches the data file and summarizes the data accumulated in the program DROSTOXDATA.FR (1) for a specified subgroup of a given run number selected by the user. This program uses the subroutines RDDROSDATA.FR and STRING.FR (Appendix B and C) to read the data sequentially and accumulates the values necessary for the number of lethal, non-lethal, and total tests performed for each brood, and grand total for the specified run number. The program also calculates the mean mutation frequency and its variance and displays them as percentages. The detection of three error conditions is provided to see (a) if more than 25 records are present for the specified subgroup within the run; (b) if the file name under which the data from the DROSTOXDATA are stored is present; (c) if the specified subgroup is present.

The program DRUNSUM.FR (2) requests the following information from the user (Figure 1):

- The name of the file to be searched.
- The run number to be searched.
- The group designator of the male code number (this specifies if the male was from the control, positive, or test compound).

The second program is part of the BMDP statistical software[TM] (3). Instructions for its use are given in this statistical package. This package analyzes the data in a 2 x 2 table and two statistics are used, the Yates corrected chi-squares and Fisher's Exact test. The Yates correction is intended to improve the approximation to the chi-square distribution. The Fisher's Exact test is computed when the

minimum value is less than 20. Both 1-tail and 2-tail probabilities are tested. However the 2-tail probability for Fisher's Exact test should be used.

The program requests the following information from the user:

●Enter the number of lethal, lethal mutations and non-lethal mutations resulting from the negative control.

●Enter the number of lethal mutations and the number of non-lethal mutations resulting from the concurrent test compound.

The computer will then type the values and probabilities associated with the Yates corrected chi-square and the Fisher's Exact test.

COMMENT

By utilizing these programs and the subroutines presented in this report, the raw data may be summarized and statistical analysis performed.

CONCLUSION

None

RECOMMENDATION

None

REFERENCES

1. Jederberg WW, Powers NR. Computer-assisted data storage and retrieval in mutagenicity testing II. The Drosophila melanogaster sex-linked recessive lethal assay. Technical Note No. 82-42-TN. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1982.

2. LAIR SOP-OP-STX-63, Execution of FORTRAN V Program DRUN: 14 June 1982.

3. Brown BM. Frequency tables. In: Dixson WJ, Brown MB, Engleman L, Frane JW, Hill MA, Jennrich RI, Toporek JD, eds. BMDP statistical software, Berkeley: University of California Press, 1981: 1943-161.

SAMPLE RUN OF DRUN

) DRUN
The computer will type:
    NAME OF FILE TO BE SEARCHED: DROSDATA

The computer will type:
    ****DROSOPHILA RECORD SEARCH AND SUMMARY****
    PLEASE ENTER RUN NUMBER TO BE SUMMARIZED: 24

The computer will type:
    ENTER GROUP TO BE SEARCHED FOR:
    (T1, T2, P1 or C1 ETC. ALLOWED): C1

The computer will type:
    SUMMARY TOTALS
    RUN NUMBER: 24
    GROUP TYPE: C1
                          BROOD DATA
LETHALS: 0   1   0   1
NONLETHALS: 596   524   547   547

GRAND TOTAL LETHALS: 2
GRAND TOTAL NONLETHALS: 2214

TOTAL RECORDS PROCESSED: 25

TOTAL TESTS REPRESENTED: 2216
MEAN MUTATION RATE: .08000%, WITH VAR. .07667%

    The computer will then display ")", the user may then return to
the program for the next file to be searched or log off.

                    Figure 1. Sample Run of DRUN
            (Underlined entries are sample user inputs)

Page

APPENDIX A

```
C *** PROGRAM NAME:        DRUNSUM.FR
C *** WRITTEN BY:          WARREN W. JEDERBERG
C *** PURPOSE:             TO SUMMARIZE THE DATA FROM SPECIFIED
C ***          RUNS IN DROSDATA.
C *** THE SUBROUTINE RDDROSDATA IS USED TO READ EACH RECORD
C *** VARIABLE DEFINITIONS
C      N   =NUMBER OF RECORDS
C      FBL =FIRST BROOD LETHALS
C      FBNL=FIRST BROOD NONLETHALS
C      STL =SECOND BROOD LETHALS
C      STNL=SECOND BROOD NONLETHALS
C      TTL =THIRD BROOD LETHALS
C      TTNL=THIRD BROOD NONLETHALS
C      FTL =FOURTH BROOD LETHALS
C      FTNL=FOURTH BROOD NONLETHALS
C
C      GTT = GRAND TOTAL TESTS
C      GTL =GRAND TOTAL LETHALS
C      GTNL=GRAND TOTAL NONLETHALS
C      TMR =TOTAL MUTATION RATE
C      V1MR=ACCUMULATED MUTATION RATES SQUARED
C      NNR =DESIGNATED RUN NUMBER (USER INPUT AT RUN TIME)
C      NS  =SUBGROUP DESIGNATOR   (USER INPUT AT RUN TIME)
C      RMR =MEAN MUTATION RATE
C
C      VARIABLES MENTIONED IN THE COMMON STATEMENT ARE DEFINED IN
C      THE FORTRAN V PROGRAM DROSTOXDATA.
       INTEGER NR,MNUM,CNAM,BFF,BFL,BFNL,BSF,BSL,BSNL,BTF,BTL,BTNL,
      1BLF,BLL,BLNL,TF,SL,ML,TL,TNL,TT,SFNAM

       DIMENSION NS(2),SFNAM(20)
       REAL MR

       COMMON/DREC/NR,MNUM(6),CNAM(6),BFF,BFL,BFNL,BSF,BSL,BSNL,BTF,BTL,
      1BTNL,BLF,BLL,BLNL,TF,SL,ML,TL,TNL,TT,MR
```

APPENDIX A
(continued)

```
      LOGICAL EOF

C *** SET ACCUMULATORS TO 0.00
      N=0
      FBL=0
      FBNL=0
      STL=0
      STNL=0
      TTL=0
      TTNL=0
      FTL=0
      FTNL=0
      GTT=0
      GTL=0
      GTNL=0
      TMR=0
      V1MR=0

C *** INITIALIZE EOF
      EOF=.FALSE.

C *** GET FILE NAME TO CHECK FOR THE DATA
      WRITE (10,220)
  220 FORMAT ( '"NAME OF FILE TO BE SEARCHED: ",Z)
      READ (11,210) SFNAM
  210 FORMAT (2CA2)
      CALL STRING (SFNAM,20)
      OPEN 1,SFNAM,ATT="SIB",ERR=100
      READ (1,5)
    5 FORMAT (/)
      GO TO 120
  100 WRITE (10,6) SFNAM
    6 FORMAT (//,"**** ERROR **** ERROR **** ERROR ****",/,
     14X,"FILE ",20A2," NOT FOUND . . . .")
      STOP
  120 CONTINUE
C *** PRINT HEADER AND GET RUN NUMBER
      WRITE (10,1)
    1 FORMAT (/,10X,"**** DROSOPHILA RECORD SEARCH AND SUMMARY ****",/,
     110X,"PLEASE ENTER RUN NUMBER TO BE SUMMARIED: ",Z)

      ACCEPT NNR
```

APPENDIX A
(continued)

```
C *** GET GROUP DESIGNATOR
   99 CONTINUE
      WRITE (10,3)
    3 FORMAT (/,10X,"ENTER GROUP DESIGNATOR TO BE SEARCHED FOR :",/,
     110X,"(T1,T2,P1,OR C1 ETC. ALLOWED):",Z)
      READ (11,4)(NS(I),I=1,2)
    4 FORMAT (2A1)
      IF (NS(1).NE."C ".AND.NS(1).NE."P ".AND.NS(1).NE."T ") GO TO 99
C *** READ DATA RECORDS
  140 CALL RDDROSDATA (EOF)
      IF (EOF) GO TO 225
      IF (NR.NE.NNR) GO TO 140
      IF (NS(1).NE.MNUM(1)) GO TO 140
      IF (NS(2).NE.MNUM(2)) GO TO 140
      N=N+1
      IF (N.GT.25) GO TO 500
      FBL=FBL+BFL
      FBNL=FBNL+BFNL
      STL=STL+BSL
      STNL=STNL+BSNL
      TTL=TTL+BTL
      TTNL=TTNL+BTNL
      FTL=FTL+BLL
      FTNL=FTNL+BLNL
      GTL=GTL+TL
      GTNL=GTNL+TNL
      GTT=GTT+TT
      TMR=TMR+MR
      V1MR=V1MR+(MR**2)
      GO TO 140

C *** IF N>25 ERROR
  500 CONTINUE
      WRITE (10,7)
    7 FORMAT (/,"*** ERROR *** ERROR *** ERROR ***",/,
     14X,"MORE THAN 25 RECORDS FOUND . . . ")
      STOP
```

APPENDIX A
(continued)

```
  225 CONTINUE
C *** IF NO SPECIFIED RECORDS FOUND
      IF(N.NE.0.0) GO TO 300
      WRITE (10,9) NNR,NS,SFNAM
    9 FORMAT (/,"*** ERROR *** ERROR *** ERROR ***",/,
   14X,"RUN NUMBER:",2X,I4,1X,"GROUP:",1X,2A1,/,
   24X,"NOT FOUND IN ",1X,20A2," . . .")
      STOP

  300 CONTINUE
C *** CALCULATE MUTATION RATE AND VARIANCE
      RMR=(TMR/N)
      VMR=(V1MR-((TMR**2.0)/N))/(N-1)

C *** WRITE OUT TOTALS
      WRITE (10,8) NNR,NS,FBL,STL,TTL,FTL,FBNL,STNL,TTNL,FTNL,
   1GTL,GTNL,N,GTT,RMR,VMR

    8  FORMAT (20X," SUMMARY TOTALS:",/,
   110X,"RUN NUMBER:",2X,I4,/,
   210X,"GROUP TYPE:",2X,2A1,/,
   320X,"BROOD DATA:",/,
   410X,"LETHALS:",4(5X,I3),/,
   57X,"NONLETHALS:",4(5X,I3),//,
   610X,"GRAND TOTAL LETHALS:",5X,I5,/,
   77X,"GRAND TOTAL NONLETHALS:",5X,I5,//,
   810X,"TOTAL RECORDS PROCESSED:",5X,I2,//,
   910X,"TOTAL TESTS REPRESENTED:",5X,I5,/,
   110X,"MEAN MUTATION RATE:",1X,F10.5,"%, WITH VAR.",1X,F10.5,"%",/)
      CLOSE 1
      END
```

APPENDIX B

```
      COMPILER NOSTACK
C *** PROGRAM NAME:          RDDROSDATA.FR
C *** WRITTEN BY:            WARREN JEDERBERG
C *** PURPOSE:               TO READ DATA FOR MAKING REPORTS
C ***           FOR THE SLRL-DROSOPHILA ASSAY

      SUBROUTINE RDDROSDATA (EOF)
      COMMON/DREC/NR,MNUM(6),CNAM(6),BFF,BFL,BFNL,BSF,BSL,BSNL,
     1BTF,BTL,BTNL,BLF,BLL,BLNL,TF,SL,ML,TL,TNL,TT,MR

      INTEGER NR,MNUM,CNAM,BFF,BFL,BFNL,BSF,BSL,BSNL,BTF,BTL,BTNL,
     1BLF,BLL,BLNL,TF,SL,ML,TL,TNL,TT

      REAL MR

      LOGICAL EOF

      READ (1,100, END= 120) NR,MNUM,CNAM,BFF,BFL,BFNL,BSF,BSL,BSNL,
     1BTF,BTL,BTNL,BLF,BLL,BLNL,TF,SL,ML,TL,TNL,TT,MR

  100 FORMAT(I4,1X,6A1,1X,6A1,4(1X,3I2),1X,I2,1X,3I2,
     12(1X,I3),1X,F6.2)
      RETURN

  120 EOF = .TRUE.
      RETURN
      END
```

APPENDIX C

```
C --- TO CONVERT FILE NAMES TO USE IN OPEN STATEMENTS
      SUBROUTINE STRING(LINE,LLEN)
      COMPILER STATIC
      DIMENSION LINE (LLEN)
      DO 100 I=1,LLEN
      IF (LINE(I).EQ."   ") LINE(I)=0
      IF (FLD(LINE(I),9,16).EQ.FLD("   ",9,16))FLD(LINE(I),9,16)=0
  100 CONTINUE
      RETURN
      END
```

END

FILMED

8